AMENDED CLAIMS

[received by the International Bureau on 06 August 2001 (06.08.01);
original claims 8, 17, 37, 47-49 amended; claims 51-52 added;
remaining claims unchanged (5 pages)]

1. A plastid transformation and expression vector which comprises an expression cassette comprising as operably linked components, a 5' part of the plastid DNA sequence inclusive of the spacer sequence, a promoter operative in said plastids, a selectable marker sequence, at least one DNA sequence encoding at least a portion of an immunoglobulin chain, a transcription termination region functional in said plastid and the 3' part of the plastid DNA sequence.

2. A plastid transformation and expression vector of claim 1 wherein the immunoglobulin chain comprises a heavy chain.

3. A plastid transformation and expression vector of claim 1 wherein the immunoglobulin chain comprises a light chain.

4. A plastid transformation and expression vector of claim 1 wherein the immunoglobutin chain comprises both a heavy and a light chain.

5. A plastid transformation and expression vector of claim 1 wherein the immunoglobulin chain comprises a single-chain variable fragment (scFv).

6. A plastid transformation and expression vector of claim 1 wherein the immunoglobulin chain comprises a heavy chain constant region fused to an operative ligand.

7. A plastid transformation and expression vector of claim 4 wherein the heavy and light chains are separated by a linker comprising an intervening stop codon and ribosome binding site.

8. A plastid transformation and expression vector of claim 1 which comprises an expression cassette comprising as operably linked components, a 5' part of the plastid DNA inclusive of the spacer sequence, a promoter operative in said plant cell plastids, a selectable marker sequence, a J chain coding sequence, a transcription termination region functional in said cells and the 3' part of the plastid spacer sequence.

9. A vector of claim 8 which comprises a secretary component with the J chain.

10. A vector of claim 9 in which the secretary component and the J chain are separated by a linker which comprises an intervening stop codon and a ribosome binding site.

11. A vector of claim 4 which comprises further a J chain and a secretary component, thereby producing secretary immunoglobulin A (SigA).

12. A plastid transformation and expression vector of claim 1 wherein a 5' part trnA gene is a plastid flanking sequence, the promoter is a 16S rRNA promoter (Prrn) driving the selectable marker gene aadA conferring resistance to spectinomycin, the psbA 3' region is a

transcription termination region functional in said cells, and the trnI gene is the 3' part of the plastid spacer, thereby defining the pLD vector.

13. A composition comprising of polypeptide multimer and plant material, wherein said multimer comprises an immunologically active immunoglobulin molecule produced from a DNA sequence integrated into the genome of a plant plastid.

14. The composition of claim 13 wherein said immunoglobulin molecule is nonglycosylated.

15. The composition of claim 13 wherein the DNA sequence encoding said immunoglobulin molecule comprises at least one sequence encoding a glycosylation signal sequence.

16. The composition of claim 14 wherein the DNA sequence encoding said immunoglobulin molecule comprises at least one sequence encoding a glycosylation signal sequence.

17. The composition of claim 13 wherein said immunoglobulin molecule is non-glycosylated.

18. A plant plastid comprising a DNA sequence encoding a polypeptide multimer encoding an immunologically active immunoglobulin molecule.

19. A plant cell comprising at least one plastid of claim 18.

20. A plant comprising at least one plastid of claim 18.

21. A plant plastid preparation comprising plastids of claim 18.

22. A composition comprising a polypeptide multimer and plant material, wherein said multimer comprises an immunologically active non-glycosylated immunoglobulin prepared from plant plastids of claim 18.

23. The composition of claim 13 wherein the polypeptide multimer further comprises a J chain.

24. The composition of claim 13 wherein the polypeptide multimer further comprises a secretary component.

25. The composition of claim 13 wherein the polypeptide multimer further comprises a J chain and secretary component.

26. The composition of claim 17 wherein the polypeptide multimer further comprises secretary component.

27. The composition of claim 17 wherein the polypeptide multimer further comprises a J chain and secretary component.

28. A method for introducing DNA encoding immunoglobulin genes into a plastid, said method comprising: introducing a plant cell with a plastid expression vector adsorbed to a microprojectile, said plastid expression vector comprising as operably linked components, a DNA sequence containing at least one plastid replication origin functional in a plant plastid, a transcriptional initiation region functional in said plant plastid, at least one heterologous DNA sequence encoding at least a portion of an immunoglobulin chain, and a transcriptional termination region functional inlaid cells, whereby said heterologous DNA is introduced into plastid in said plant cell.

29. The method of claim 28 wherein the immunoglobulin chain comprises a heavy chain.

30. The method of claim 28 wherein the immunoglobulin chain comprises a light chain.

31. The method of claim 28 wherein the immunoglobulin chain comprises both a heavy chain and a light chain.

32. The method of claim 28 wherein the immunoglobulin chain comprises a singlechain variable fragment (scFv).

33. The method of claim 28 wherein the immunoglobulin chain comprises a heavy chain constant region fused to an operative ligand.

34. The method of claim 28 wherein said plastid expression vector further comprises DNA sequences encoding a J chain.

35. The method of claim 28 wherein said plastid expression vector further comprises DNA sequences encoding a secretary component.

36. The method of claim 28 wherein said plastid expression vector further comprises DNA sequences encoding a J chain and a secretary component, thereby producing secretary immunoglobulin (SigA).

37. A plastid transformation and expression vector which comprises an expression cassette comprising an operably linked components, a promoter operative in plant plastids, a selectable marker sequence, immunoglobulin chain coding sequences, a transcription termination region functional in said cells.

38. A plastid transformation and expression vector of claim 37 wherein the immunoglobulin chains comprise heavy chains and light chains.

39. A plastid transformation and expression vector of claim 38 which comprises covalent boding between the chains, into immunologically active immunoglobulins in the plastid.

40. A plastid transformation and expression vector of claim 39 wherein the heavy and light chains are separated by a linker comprising an intervening stop codon and ribosome binding site.

41. A plastid transformation and expression vector which comprises an expression cassette comprising an operably linked components, a promoter operative in plant cell plastids, a selectable marker, a J chain coding sequence, a transcription termination region functional in said cells.

42. A vector of claim 41 which comprises a secretary component with the J chain.

43. A vector of claim 42 which the secretary component and the J chain are separated by a linker which comprises an intervening stop codon and a ribosome binding site.

44. A vector of claim 38 which comprises further a J chain and a secretary component, thereby producing secretary immunoglobulin A (SigA).

45. A plastid transformation and expression vector of claim 44 which comprises in addition that the light chains are four identical light chains, and the heavy chains are four chains.

46. A plastid transformation and expression vector of claim 38 wherein the promoter is a 16S rRNA promoter (Prm) driving the selectable marker gene aadA conferring resistance to spectinomycin, and the psbA 3' region is a transcription region functional in said cells, thereby defining the pZS vector.

47. The stably transformed plant which has been transformed by the vector of claim 37 and the progeny thereof.

48. The progeny of the stably transformed plant of claim 47, wherein such progeny are seeds.

49. The plant of claim 47, wherein the plant is tobacco.

50. A universal plastid transformation and expression vector which comprises an expression cassette comprising as operably linked components, a 5' part of the plastid spacer sequence, a promoter operative in said plant cell plastids, a selectable sequence marker, at least one DNA sequence encoding at least a portion of a immunioglobulin chain, a transcription termination region functional in said cells and the 3' part of the plastid spacer and flanking each side of the expression cassette, flanking DNA sequences which are homologous to a DNA sequence inclusive of a spacer sequence conserved in the plastid genome of different plant species, whereby stable integration of the heterologous coding sequence into the plastid genome

of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target plastid genome.

51. The stably transformed plant which has been transformed by the vector of claim 41 and the progeny thereof.

52. The progeny of the stably transformed plant of claim 51, wherein such progeny are seeds.